Gut

Leading article

Hydrogen sulphide: a bacterial toxin in ulcerative colitis?

The cause of ulcerative colitis (UC) is unknown but it is likely to depend on an interaction between genetic factors, which may determine the immune response or the expression of enzymes that control intracellular metabolism, and environmental factors, such as diet and the nature of the bacterial flora. Ultimately, these factors result in the breakdown in the integrity of the colonic epithelial cell barrier and the perpetuation of inflammation. This review focuses on events in the colonic lumen that are largely controlled by environment, including the bacterial conversion of dietary substrates to hydrogen sulphide and the metabolic consequences for the colonic mucosa.

Metabolism of the colonic epithelial cell in health

The principal products of fermentation, the short chain fatty acids (SCFAs) are essential to the metabolism of the colonic epithelial cell and maintenance of normal mucosal function.[1] Fermentation is largely the process of breakdown of dietary and endogenous carbohydrates by anaerobic bacteria in the colon and is driven by the amount and type of substrate, most of which has escaped digestion in the small bowel. The end products are SCFAs, principally acetic, propionic and butyric acids, the gases CO_2, methane and hydrogen and biomass. SCFAs, especially butyrate, provide the colonic epithelial cell with approximately 70% of its energy.[2–4] In contrast, epithelial cells of the small bowel preferentially use glucose and glutamine as respiratory fuels,[5 6] a factor that may be of importance in understanding the pathogenesis of UC, a disease process confined entirely to the large bowel.

Impaired cellular metabolism in UC

Roediger was the first to show that butyrate oxidation was significantly impaired in active and quiescent UC.[7] This finding has been confirmed[8] and extended to include macroscopically and histologically 'normal' colonic mucosa in patients with UC.[9 10] These changes in β-oxidation are primary and precede the onset of overt colitis, a proposal supported by the induction of experimental colitis in rats by rectal instillation of 2-bromo-octanoate,[11] a specific inhibitor of β-oxidation. Based on the hypothesis that epithelial cell butyrate 'deficiency' might be important in the pathogenesis of UC, a number of clinical trials of topical SCFA therapy in distal UC have been undertaken in an attempt to overcome this partial metabolic block by 'mass action'. The data on clinical responses are generally promising,[12–15] again supporting a role for defective SCFA metabolism in UC.

Metabolic effects of reducing sulphur compounds

Roediger and colleagues have shown the potential for naturally occurring agents[16–18] within the colonic lumen to reproduce the characteristic biochemical lesion observed in colonic tissue from UC patients. In experiments using healthy human colonocytes,[19] reducing sulphur compounds at a concentration of 2 mmol/l caused selective and potentially reversible inhibition of butyrate oxidation by 75% in the distal colon and 43% in the ascending colon. The order of suppression of fatty acid oxidation was hydrogen sulphide>methanethiol>mercaptoacetate. It is possible that these agents, most potently hydrogen sulphide (HS^-), may be involved in pathogenesis. Similar studies using rat colonocytes[20] with HS^- at 0·1–0·5 mmol/l, concentrations that are physiological within the colonic lumen,[21–25] have confirmed these findings. The point at which HS^- is inhibitory in the metabolism of butyrate seems to be proximal to NAD linked oxidation,[26] at the level of butyryl-CoA dehydrogenase.[20] Reducing sulphur compounds may also diminish butyrate oxidation in the colonic epithelium as mercaptans compete with butyrate for uptake by the SCFA ion exchange transporter.[27]

Toxicity of hydrogen sulphide

Hydrogen sulphide has a number of potentially adverse effects that could play a part in the pathogenesis of UC. A reversible increase in epithelial permeability and loss of barrier function by HS^- has been shown in oral mucosa[28] but to what extent this may be due to a breakdown of the polymeric structure of mucin from cleavage of disulphide bridges is unknown. Incubation of healthy human colonic biopsy specimens with 1 mM HS^- is associated with an increase in upper crypt labelling index, whereas co-incubation with 10 mM butyrate confers protection.[29] Thus, HS^- effectively reproduces the abnormalities in cell proliferation observed in the crypt epithelium in UC, perhaps as a result of DNA hypomethylation.[30] Perfusion of the colon in anaesthetised rats with NaHS[31] at physiological

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concentrations produces dose related apoptosis, goblet cell loss, crypt architectural distortion, and superficial mucosal ulceration. Furthermore, the anaerobic conditions in the colon favour the splitting of structural disulphide bonds of complement factor 3 (C3) by HS^- with a resultant compromise in opsonisation potential.[32] Functionally, this effect is translated into an inhibition of the capacity of polymorphonuclear leucocytes to phagocytose and kill encapsulated strains of bacteria. Such immunomodulatory effects of HS^- may be important in promoting bacterial translocation[33] and systemic endotoxaemia[34] associated with UC. This evidence is indicative that HS^- may act from the lumen to produce toxic effects on the colonic epithelium. HS^-, in turn, arises as result of bacterial metabolism.

Gut bacteria and animal models of colitis

The limitation of UC to the large intestine and the intimate association of its epithelial surface with a complex ecosystem comprising $>10^{11}$ bacterial cells/g contents, has long fuelled speculation about a role for the luminal gut flora in pathogenesis. Studies so far have failed to identify a bacterial pathogen[35] or provide evidence for significant dysbiosis of the normal colonic flora.[36–38] Experimental models of colitis have provided new insight by consistently demonstrating an obligate requirement for the presence of normal gut flora in the genesis of intestinal inflammation. Interleukin 2 knockout mice, for example, have recently been developed[39] which, despite an absence of pathogens in the gut, acquire spontaneous colonic inflammation with similar pathological and clinical features to human UC. Germ free interleukin 2 knockout mice, however, do not develop inflammatory bowel disease, although those raised in a specific pathogen free environment slowly acquire subclinical histological lesions. Similar findings in other transgenic animals have also been reported.[40,41]

Before these animals with immunological gene defects had been studied, considerable interest was generated by animal models in which colitis could be induced experimentally by ingestion of sulphated, but not unsulphated, polysaccharides. Degraded carrageenan, sodium lignosulphate, and sulphated amylopectin, when fed orally in drinking water to guinea pigs and rabbits, produced clinical and pathological features resembling human UC.[42,43] Pretreatment of these animals with metronidazole prevented the development of colitis, whereas antimicrobials active only against Gram positive organisms or coliforms failed to attenuate the disease process.[44] Delayed treatment with metronidazole, however, until after colitis was established, conferred no protection. In subsequent work,[45] administration of carrageenan to germ free guinea pigs did not result in typical lesions, but when these animals were conventionalised with a normal flora colitis was induced during carrageenan challenge. More recently, experimental acute and chronic colitis has been induced in mice and hamsters using dextran sulphate sodium,[46,47] with similar protective effects by metronidazole pre-treatment.[48] These data show that the fermentation of sulphated dietary substrates by a metronidazole sensitive component of the faecal flora yields a toxic product, hitherto undefined, that causes colonic inflammation and ulceration.

Sulphate reducing bacteria and production of hydrogen sulphide

The potential significance of these animal models of colitis is now emerging following identification in the rumenal[49,50] and colonic[51–55] contents of sulphate reducing bacteria (SRB), anaerobes uniquely capable of reducing inorganic sulphate to hydrogen sulphide.[56] It is noteworthy that *Desulfovibrio* species (a group of SRB) share 87·5–91% homology with the 16S rRNA sequence of a curved intraepithelial bacillus consistently associated with intestinal lesions in animals with proliferative bowel disease.[57,58] Inoculation of this bacterium into germ free animals requires the coexistence of other intestinal flora to induce disease.[59,60] Attempts to grow this bacterium on sulphate containing media have been unsuccessful,[57] however, and a recent histochemical study showed no antigenic cross reactivity with desulfovibrios isolated from patients with UC.[61]

SRB utilise the sulphate or sulphite ion as a terminal electron acceptor for the dissimilation of reduced organic substrates, principally SCFAs or molecular hydrogen, produced during colonic fermentation.[62] Sulphide is released into the acidic luminal environment and at this pH ($pK_a=7·04$) HS^- is hydrolysed to biologically active free H_2S.[63] Levels of sulphide in faeces are partly related to the presence of SRB[21] and are greater in the distal rather than the proximal colon.[64] Significant numbers of SRB (10^7–10^{11}/g wet weight faeces) grow in faecal samples of subjects without detectable breath methane but in methanogenic subjects SRB are present in lower numbers ($\leq 10^5$/g wet weight faeces).[65] These data suggest that competition for hydrogen between SRB and methanogenic bacteria occurs in the colon, a mechanism well described for aquatic ecosystems.[56]

Influence of sulphate availability on hydrogen metabolism

The physiological basis for the competition for hydrogen between SRB and methanogenic bacteria is largely dependent on the availability of sulphate.[66] Addition of sulphate and sulphated mucopolysaccharides to mixed faecal slurries containing metabolically active SRB results in the stimulation of sulphide production and reduction of methanogenesis.[67,68] In vivo, dietary supplementation with sodium sulphate results in the inhibition of methanogenesis and the growth of SRB in 50% of subjects.[69] In ruminants, dietary supplementation with sulphate is associated with a linear increase in the amount of hydrogen sulphide in the rumen.[70] If this is excessive, acute toxicity is manifest clinically as weight loss, fever, bloody diarrhoea, and intestinal inflammation.[71]

Much of the sulphate present in the British diet is added as a preservative to prolong shelf life during manufacture of processed foodstuffs, principally as sulphur dioxide (E220) and the sulphites (E221–227), and to a lesser extent as carrageenan (E407), and is consumed by 98·6% of the population.[72] Analysis of the sulphate content of foods and beverages has found that a typical rural African diet only contains 2·7 mmol/day, whereas in Western diets there may be in excess of 16·6 mmol sulphate/day.[73] A study of sulphate absorption by the small intestine in subjects with an ileostomy has shown a threshold for dietary sulphate intake of approximately 7 mmol/day, above which a significant amount spills over into the colonic sulphate pool.[74] Sulphate intake is therefore a regulating factor for dissimilatory sulphate reduction in the colon and may be important in explaining the changing prevalence of UC in developing countries[75] as well as determining apparently spontaneous shifts in H_2 consuming bacteria over time.[76]

What, therefore, is the relation between net production of hydrogen sulphide by the colonic flora and UC?

SRB and hydrogen sulphide production in patients with UC

Three studies have investigated the growth and activities of SRB in patients with UC and have reported almost universal faecal carriage.[77–79] Gibson *et al*[78] reported that 92% of SRB isolated belonged to the genus *Desulfovibrio* compared with 66% in healthy controls, and that a strain of *Desulfovibrio desulfuricans* from a patient with UC was better able to withstand fast turnover times in continuous culture, which mimic the rapid transit of diarrhoea, than a control strain. In another study[79] we observed that total viable counts of SRB were higher in faeces of patients with active compared with quiescent disease. The metabolic activities of the bacteria were not uniform, however, with approximately 30% of colitic faecal samples containing SRB with rapid growth characteristics and high sulphate reducing activity. Similar results were seen in healthy faecal samples, although sulphate reduction rates were higher among patients with UC, particularly those with active disease (M C L Pitcher, unpublished data). For the remaining subjects, bacterial production of hydrogen sulphide seemed to be derived from other sources, most probably sulphur amino acid fermentation.

Recent work has shown that treatment with 5-ASA drugs in patients with UC significantly lowers the concentration of sulphide in faeces[25] and faecal sulphide concentrations are significantly higher in patients with UC than healthy controls. In vitro studies demonstrated a dose response effect for 5-ASA from which the IC_{50} (5-ASA concentration producing half maximal inhibition) for sulphate reduction was extrapolated as 18·75 mM. This is similar to luminal concentrations of 5-ASA in patients taking maintenance doses of salicylate drugs.[80–82] Florin *et al* have presented data in abstract form[77] indicating that faecal concentrations of sulphide were significantly higher in patients with UC. Unfortunately there were few clinical data, which makes it difficult to interpret the results.

Theoretically, sulphide concentrations may also be lowered in the lumen by inhibition of SRB growth. In vitro tests of sensitivity, however, have shown that isolates of *Desulfovibrio desulfuricans* from UC patients are multiply resistant to antimicrobials[83] and this may partly explain the poor clinical efficacy of these agents in the treatment of UC. Alternatively, H_2S may be effectively removed from the colonic lumen via enzyme catalysed S-methylation by thiol methyltransferase.[84 85] Using thiol methyltransferase activity in erythrocyte membranes as a marker of total S-methylation capacity in vivo, we observed that enzyme activity was raised in UC patients compared with controls, significantly in those with active disease.[86] This may represent a homeostatic feedback response, with induction of gene transcription/translation by increased endogenous colonic sulphide production from gut bacteria. Such a protective mechanism in UC might be further induced by the effects of smoking habit.

An increased understanding of hydrogen sulphide metabolism within the colon will advance our knowledge relating to mechanisms of toxicity to the colonic epithelial cell and, in time, to a new approach to treatment in UC.

Conclusions

Net luminal production of hydrogen sulphide from bacterial sulphate reduction and amino acid fermentation is increased in UC. This is modulated by the availability of dietary substrates and concomitant treatment with 5-ASA drugs. Excess luminal sulphide may overburden the genetically determined capacity of mucosal detoxification systems, with resultant impairment of butyrate oxidation and the genesis of colonic epithelial inflammation.

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